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Clinical aspects related to endodontic yeast infections

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Yeasts can be detected in 7–18% of infected root canals. They are commonly associated with persistent cases of apical periodontitis, but yeasts can also be isolated in primary apical periodontitis. Their relative proportion of the root canal flora is low, usually below 1%. Therefore, yeast detection with conventional microbiological methods may be difficult. Selective culture media such as Sabouraud dextrose agar combined with cultivation from undiluted sample should be used for primary isolation of yeasts in endodontic infections. Preliminary identification of yeasts is based on typical colony and cellular morphology and a positive catalase test. Detection by molecular biological methods is sensitive, but may occasionally also give false-positive results. *Candida albicans* is the most commonly detected yeast species from infected root canals. It is typically found together with Gram-positive bacteria such as streptococci, but can also be isolated in pure culture, which is an indication of its pathogenicity. A variety of virulence factors enable *C. albicans* to adhere to and penetrate into dentine. Furthermore, *C. albicans* tolerates harsh ecological conditions including high alkalinity. Calcium hydroxide is generally not efficient against oral yeasts *in vitro*; the *in vivo* effectiveness of calcium hydroxide is not known. However, sodium hypochlorite, iodine compounds, and chlorhexidine have proven effective against yeasts both in experimental conditions as well as *in vivo* and may offer effective treatment possibilities against endodontic yeast infections.

Oral yeasts

Yeasts are common opportunistic pathogens in the oral cavity. Approximately one-third of individuals with no oral diseases carry yeasts (1). The most important oral yeasts belong to the genus *Candida*. *Candida albicans* is the predominant yeast species, followed by *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, *Candida guilliermondii*, *Candida kefyr*, and *Candida parapsilosis* (1). *Candida dubliniensis*, a new species closely related to *C. albicans*, is rarely found in the oral microflora of normal healthy individuals. However, epidemiological studies have shown that *C. dubliniensis* is primarily carried by and causes oropharyngeal infections in acquired immune deficiency syndrome patients (2). Other yeast genera have also been isolated from the oral cavity e.g. *Saccharomyces* spp. and *Geotrichum* spp. (3–5). However, their pathogenic importance is limited in comparison with the genus *Candida*. *C. albicans* is an adaptive microorganism that expresses various virulence factors and demonstrates different growth forms such as germ tubes, yeasts (blastospores), pseudo- and true hyphae, and chlamydospores (1, 6).

Isolation of yeasts

The relatively late appearance of strictly anaerobic bacteria in research reports of the root canal microflora can be explained by the difficulty in culturing and identifying anaerobic bacteria as compared with facultative and aerobic species. The rare reports on yeasts in endodontic literature, however, cannot be explained similarly. Yeasts, in particular those that are the most common yeast isolates in oral infections, are generally quite easy to culture and isolate. There are other reasons why yeasts may be overlooked in microbiological specimens from endodontic infections. One is a low colony-forming unit (CFU) number

as compared with bacteria. Peciuliene et al. (7) reported that yeasts constituted only <1% of the total cultivable flora in most specimens. In clinical laboratories, colonies for preparing pure cultures are usually selected from plates with 5–50 CFU after serial dilutions. Despite their presence in the original sample, yeast colonies may no longer be found on the plates used for further culturing. In addition, on non-selective media, yeasts may be disregarded as contaminants as their colony morphology on the plate resembles that of typical contaminants from the air or other sources.

The preparatory procedures for adequate sampling include mechanical cleaning of the tooth with pumice, e.g., followed by isolation with a rubber dam, and disinfection of the tooth surface and the rubber dam according to established procedures (8). Strict asepsis is crucial at each step, including disinfection of the dentine cavity before trepanation into the pulp chamber and neutralization of the disinfecting solutions before trepanation. Paper points are usually best suited for sampling the necrotic root canal. However, as some brands may be inhibitory to microbial growth (9), it is recommended that paper points that are charcoaled or chloroform washed before sterilization be used to avoid false-negative samples (7, 8, 10).

Yeasts can be best isolated from endodontic samples by using selective media for the primary isolation. Yeasts tolerate a much wider pH range than bacteria, and therefore several selective media are available for the isolation of yeasts. Sabouraud agar is a commonly used medium for the isolation of oral yeasts. The pH of the medium is quite acidic (usually 5.6), allowing the growth of yeasts and aciduric organisms, whereas most bacteria are inhibited. The pH can be further reduced by hydrochloric acid to 3.0–4.0 to prevent the growth of aciduric bacteria. Several different versions of Sabouraud agar are available on the market; Sabouraud dextrose agar is the most frequently used in the isolation of oral yeasts. Sometimes, bacterial growth is further prevented by additives such as chloramphenicol, streptomycin, novobiocin, penicillin, or other antibiotics, but this is not necessary for effective isolation of yeasts from endodontic infections. However, it is important that as much of the undiluted sample is plated as possible to ensure detection of yeasts in cases where their total CFU is low (7).

Tryptic soy-serum–bacitracin–vancomycin (TSBV) medium is a selective medium used primarily for the isolation of *Actinobacillus actinomycetemcomitans* and closely related *Haemophilus* spp. (11). Because of its high discriminative power against most other bacteria, TSBV plates have often also been used for the primary isolation of oral yeasts, which grow well on this medium (12). However, high numbers of Gram-negative enteric rods, which also grow on TSBV plates, can hamper the detection of yeast colonies, which are typically less numerous if both microbial groups are present. Therefore, it may be recommended to use Sabouraud dextrose medium, or use both media. TSBV medium is clearly more expensive because of the added serum. Unfortunately, there are no comparative studies with a large number of clinical samples on the effectiveness of the two media in isolating oral yeasts. It is the experience of the authors that when yeasts are the primary research interest, Sabouraud plates should be the first choice. It should be noted that yeasts also grow on some other selective media, which target aciduric bacteria, such as Rogosa agar (for the isolation of lactobacilli) and MS agar (for the isolation of *Streptococcus mutans* and other related oral streptococci). However, these media should not be used specifically for the isolation of oral yeasts. Oral yeasts grow best in air at 37°C in humid conditions. They grow poorer in air plus 5% carbon dioxide (optimal for many facultative bacteria), and may even fail to grow under anaerobic conditions in an anaerobic cabinet or anaerobic jar, e.g.

Identification of yeasts

Yeasts grow rapidly on the plates and the colonies can be picked for biochemical and other tests from overnight cultures. Yeast colonies are usually white or yellowish, typically two to three times larger than bacterial colonies. They may have the appearance of staphylococcal colonies, but the surface of the colony is usually clearly drier than that of staphylococci. The isolated colonies can be readily identified as oral yeasts by a positive catalase test (vigorous 'bubbling' when dropped into freshly made 3% hydrogen peroxide) and by typical cellular morphology in wet mount specimens in phase contrast microscopy (Fig. 1). In Gram stain oral yeasts stain intensively Gram-positive, and the cell size (2.5–5 μm), which is several times larger than the size of bacteria (diameter 0.3–0.7 μm), confirms the preliminary identification.

C. albicans, which is the by far the most common yeast in oral infections, including endodontic

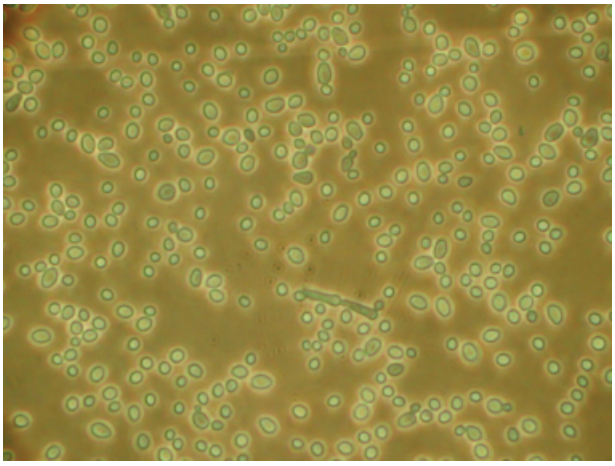

Fig. 1. Phase contrast microscopy picture of *Candida albicans* cells. Typical cell morphology of yeast cells can be seen.

infections, can be reliably identified by a germ tube test after preliminary identification (as yeast). The germ tube test for *C. albicans* is performed by suspending a low concentration of yeast cells in small volume of serum; commercial germ-tube media are also available. After 2–3 h of incubation, a wet mount is prepared and examined by phase contrast microscopy at × 400. A germ tube (without septal constrictions) is narrower but several times longer than the parent cell (13).

For routine diagnostic purposes, oral yeasts can be identified by using commercially available identification kits. The individual tests in such kits are measuring fermentation and/or assimilation of various carbohydrates and production of a variety of glycosidase and aminopeptidase enzymes (14–16). While most *Candida* spp. and other oral yeast taxa can be reliably identified by these test systems, *C. dubliniensis* remains a challenge. However, the new kits can with relative certainty differentiate also between *C. albicans* and *C. dubliniensis* (15, 16).

Kurzai et al. (17) reported that the formation of chlamydospores on Staib agar was found with *C. dubliniensis* strains but not with *C. albicans*, suggesting that this is an easy to do, reliable test for differentiating between the two species. The authors reported a 100% sensitivity and specificity of the chlamydospore test on Staib agar, identical to rDNA sequencing and discriminative polymerase chain reaction (PCR), which both had been previously shown to be reliable methods to differentiate between the two closely related species (18). Recently, Khan et al. (19) showed that *C. albicans* and *C. dubliniensis* can be identified by differences in colony morphology on sunflower seed agar. The results of the phenotypic tests were confirmed by semi-nested PCR amplification of rDNA using species-specific primers, followed by direct sequencing of the product (19). The suitability of sunflower seed agar in the differentiation of *C. albicans* and *C. dubliniensis* has also been shown by Al Mosaid et al. (20).

Chromogenic media are based on selective yeast media supplemented with special chromogenic substrates to give a specific color to the different yeast species. Comparative studies indicate that these media can be used even for primary isolation of yeasts from clinical samples (21, 22). However, while chromogenic media are useful for preliminary identification of yeasts, they cannot differentiate between all species, including *C. albicans* and *C. dubliniensis.*

Phenotypic characterization is in many cases a valid and sufficient method for the identification of oral yeasts. However, sometimes it may be necessary to use methods based on the DNA analysis of the isolated yeast species and strains. Such methods include arbitrarily primed PCR (23), species-specific DNA probes (24, 25), and use of real-time LightCycler PCR (26, 27). Recently, Luo & Mitchell (28) reported successful use of multiplex PCR for rapid identification of pathogenic fungi directly from cultures. The authors were able to amplify DNA directly from the yeast colonies, thus avoiding the steps needed for purifying genomic DNA.

Yeasts in root canal infections

Throughout the past decades, it has been well known that yeasts can be isolated from infected root canals (10, 29–41). The occurrence of yeasts reported in infected root canals varies between 1% and 17% (30, 31, 42). In a study with an extensive material of 967 microbiological samples taken from persistent cases of apical periodontitis, yeasts were isolated from 7% of culture-positive samples (41). The identification of yeasts was carried out with conventional clinical laboratory procedures. Almost all isolates belonged to the genus *Candida*, and *C. albicans* was the predominant species. *C. glabrata*, *C. guilliermondii*, *C. inconspicua*, and *Geotrichum candidum* were also isolated.

In a study of 100 previously root-filled teeth with apical periodontitis, Molander et al. (10) isolated *C. albicans* in three root canals (3%). However, selective

media for yeasts were not used in this study. The authors also investigated the microbial flora in 20 cases of previously root-filled teeth with an unsatisfactory root filling but no periapical lesion and found *C. albicans* in two root canals. Sundqvist et al. (43) found yeasts (*C. albicans*) from two of 24 teeth after failing endodontic treatment. Hancock et al. (44) studied the microflora in 54 root-filled teeth with persistent periapical radiolucencies. They found growth in 34 teeth; *C. albicans* was isolated from one tooth. Selective media for yeasts were not used. Pinheiro et al. (45) studied the flora in 60 root-filled teeth with persisting periapical lesion. Microorganisms were isolated from 51 teeth, and *Candida* spp. from two teeth. Also, in this study, selective yeast media were not used.

Peciuliene et al. (7) studied the occurrence of yeasts, enteric Gram-negative rods, and *Enterococcus* spp. in root-filled teeth with chronic apical periodontitis. Forty teeth were included in the study, and growth was detected in 33 teeth using conventional culturing methods including selective media for yeasts (TSBV and Sabouraud plates). Yeasts were isolated from six teeth (18% of the culture-positive teeth). All isolates belonged to the species *C. albicans*. Egan et al. (46) investigated 60 root canal samples in teeth with apical periodontitis (25 root filled, 35 untreated) using selective media (Sabouraud dextrose agar), and reported eight yeast isolates in six teeth. Six of the isolates were *C. albicans* while two were identified as *Rodotorula mucilaginosa*. A commercial biochemical test kit was used for the identification of the yeasts (46). The authors also showed that the probability to have yeasts in the root canal was 13.8 times higher, when the patient had cultivable yeasts in saliva, the difference being statistically significant. Previous root canal treatment, coronal leakage, or previous antibiotic therapy did not seem to have an association with the occurrence of yeasts. Cheung & Ho (47), using selective yeast culture media, found microbial growth in 12 of 18 teeth, two with *C. albicans*.

Although the number of studies of yeasts in endodontic infections as well as the number of cases in several of the studies is relatively low, it seems that the frequency of isolation of yeasts is less than 5% when selective yeast media have not been used. However, when selective yeast culture media have been used for primary isolation, yeasts can be found in 7–18% of the teeth. Whether there is a difference between teeth with apical periodontitis with and without previous root canal treatment remains unclear at present because of the low number of comparative studies specifically focusing on the isolation of yeasts.

Diagnostic procedures using molecular techniques have a higher sensitivity than conventional culturing techniques. Higher numbers of microbial species, including novel taxa, have been identified from root canals using PCR-based molecular detection techniques (48). In a study on randomly selected patients with periapical radiolucencies, *C. albicans* was detected by PCR technics in five out of 24 samples (21%) taken from infected root canals of teeth with primary apical periodontitis (42). In the same study, samples were also collected from 19 cases of cellulitis/abscesses using needle aspirates. All 19 samples were found to be negative for the presence of *C. albicans* DNA. Contrary to the root canal samples in the previous study, Siqueira et al. (49), using group-specific primers and PCR, found fungi in only one of 91 root canal samples taken from primary apical periodontitis.

Further studies with larger numbers of patients are necessary to confirm these findings. However, it is important to bear in mind that DNA techniques using PCR to multiply part of the microbial genome cannot discern between dead material and living organisms. Therefore, the goals of the investigation should, to some extent, guide the choice of techniques used for the detection of microorganisms in endodontic infections. When the primary goal is to find out which microorganisms are or have been present in the root canal, sensitive molecular methods may give maximum information. However, when the sample is taken to help in planning effective treatment strategy in individual cases, culture methods should be given priority, as they reveal the viability of the detected organisms.

Mixed infections

C. albicans can occasionally be found in pure culture in the root canal. However, yeasts are usually isolated in mixed infections together with bacteria. Facultative Gram-positive bacteria such as α- and non-hemolytic *Streptococcus* species are commonly found together with yeasts, whereas Gram-negative isolates are rare (41). It is possible that ecological conditions in the necrotic root canals favor the growth and coexistence of yeasts and streptococci. In addition, *C. albicans* coaggregates with a variety of streptococci such as *Streptococcus gordonii*,

Streptococcus mutans, and *Streptococcus sanguis* (50, 51), which may facilitate biofilm formation. Biofilms again are known to promote colonization and survival of individual taxa, further explaining the concomitant occurrence of yeasts and streptococci in necrotic root canals (52).

Dentine infection and virulence factors

Microscopic examination of extracted teeth associated with apical periodontitis and *in vitro* investigations on the ability of yeasts to adhere and invade dentine have increased our knowledge about yeasts in root canal infections (Figs 2–4). Nair et al. (36) provided evidence of the presence of yeast-like organisms, in addition to bacteria, in the root canal and apical foramen using light and electron microscopy. The organisms found had the typical size and cellular appearance of fungal organisms including distinct cell wall, nuclei, and budding growth forms indicating proliferation of the cells. In this study, the presence of intraradicular fungi in endodontically treated human teeth was associated with periapical lesions that persisted after root canal therapy. Further observations on bacteria and fungi using scanning electron microscopy in infected root canals and dentinal tubules associated with periapical lesions were made by Sen et al. (38). They found root canals heavily infected with yeasts in ovoid and hyphal forms. In subsequent studies, Sen et al. (39) investigated the growth patterns of *C. albicans* on native and chemically modified dental hard tissues. Yeast cells were allowed to attach to or grow on enamel, cement, and dentine, with or without previous treatment by ethylendiamine tetra-acetic acid EDTA and sodium hypochlorite. *C. albicans* was grown in the presence of serum before the experiments. The authors found no difference in the growth pattern of *C. albicans* between enamel and cement. Pretreatment with EDTA/NaOCl had no effect on the results. A biofilm consisting of yeasts and *C. albicans* hyphae was consistently detected on these two dental hard tissues. The surfaces were covered with separate, but dense colonies. In dentine specimens with a smear layer, the colonies were not distinct and presented a dense mass of yeast cells and mycelia, forming a biofilm. No dense colonies could be detected in the absence of a smear layer. In many areas, the hyphae were seen to penetrate into dentinal tubules, accompanied by single yeast cells. The observations suggested that the presence of smear layer

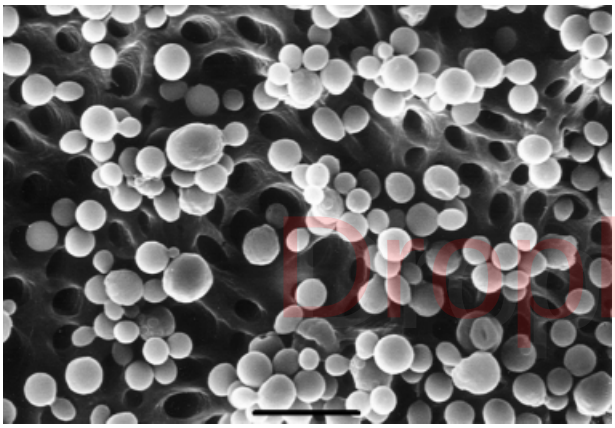

Fig. 2. Scanning electron micrograph of budding yeast cells in pure culture on root canal wall. (bar remarks 10 μm).

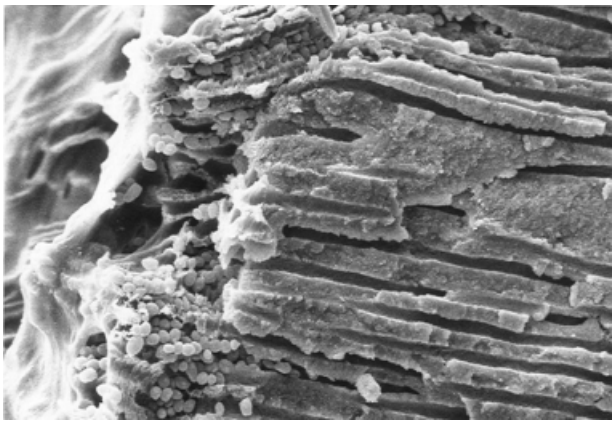

Fig. 3. Yeast infection of dentin. Some dentinal tubules are infected whereas the majority of the tubules remain free of cells.

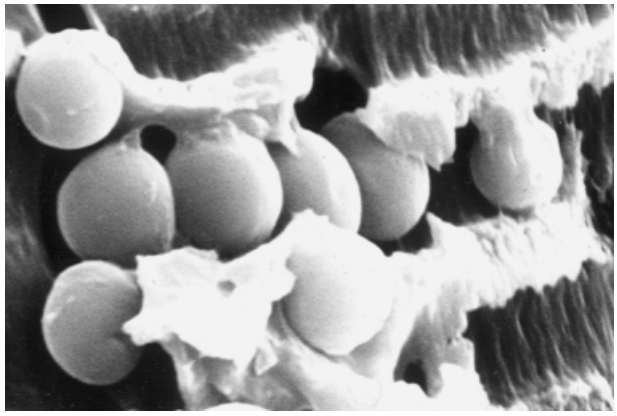

Fig. 4. *Candida albicans* adhering and invading into a dentinal tubule.

favors the growth and attachment of *C. albicans* to root dentine (40). Compared with other oral yeast species, *C. albicans* seems to have superior properties for dentine colonization and infection. Together with its predominant occurrence in the oral cavity, this may explain its isolation also from root canal infections (53).

The presence of oral yeasts in carious lesions has been known for a long time (54, 55). However, the dynamics of yeast invasion into dentinal tubules is largely unknown. Recently, an *in vitro* method was developed to study the penetration into human dentine by *C. albicans* and *Enterococcus faecalis* (56). Both macroscopic recording of the penetration through dentine discs and microscopic examination of microorganisms in dentinal tubules were performed. *C. albicans* growth into dentinal tubules was relatively weak in comparison with *E. faecalis*. However, both organisms were able to penetrate through a 2 mm thick human dentine disc. Histological preparations showed penetration of ovoid or budding yeast cells as well as hyphae into a few dentinal tubules, whereas most of the tubules remained free of cells. Continuous penetration could be seen up to a depth of 60 μm. Beyond that, single budding cells in low numbers could be found throughout the dentine specimens. It is worth noting, however, that the dentinal canals were patent throughout the specimen and not blocked by a layer of cement, e.g. A corresponding situation where the cement layer is missing is known to occur at the root tip area in chronic apical periodontitis, where resorption of some areas of root cement has been documented in histological specimens of extracted teeth or root tips after apicoectomy (57).

A characteristic feature of mycotic infections is their development when the host provides the environmental conditions and nutrients essential for attachment, growth, and reproduction of fungi. Thus, for mycoses to occur, local and/or general predisposing factors are required. The necrotic pulp space and adjacent dentine, although a harsh ecological environment, provides essentials needed for a *Candida* infection to develop. On the other hand, a variety of virulence factors such as adherence, hyphae formation, thigmotropism (contact sensing) for penetration, protease secretion, and phenotypic switching are required on the side of the pathogen. Adherence of *C. albicans* to dentine and biofilm formation are likely to increase its resistance to antimicrobial agents and changes in the pH. The capability to penetrate into dentinal tubules is based on pleomorphic growth patterns as well as thigmotropism. Because of these factors, the entire root canal system can be invaded by *C. albicans*, making its conservative elimination from dentine difficult, although this has not been shown *in vivo*. Aspartyl protease secretion and enzymes capable of degrading dentinal collagen can further improve the ability of *C. albicans* to survive in limited nutrient conditions. Spontaneous phenotypic switching may further promote the survival in changing microecological environment. These factors make *Candida* species exceptionally adaptive to tolerate environmental and nutritional changes during root canal therapy.

Periradicular yeast infections

Periapical actinomycosis is an endodontic infection, where the bacteria are residing outside the root canal system in the periapical area. Several different *Actinomyces* species have been isolated in the bacterial macrocolonies from these lesions (58–61). It has been suggested that some other bacteria may also be involved in periapical actinomycosis, although it is not known whether they represent extruded bacterial colonies during endodontic treatment or true actinomyces-like infection (61, 62).

The information available about the occurrence of yeasts in the periapical tissues of teeth with apical periodontitis is scarce. Nair et al. (36) analyzed nine therapy-resistant and asymptomatic human periapical lesions by light and electron microscopy, using block biopsies that were removed during surgical treatment of the affected teeth. The cases that required surgery represented about 10% of all of the cases that received endodontic treatment 4–10 years earlier. Six of the nine biopsies revealed the presence of microorganisms in the apical root canal. Four contained one or more species of bacteria and two revealed yeasts. However, colonies of microorganisms were not detected in the periapical area. In two other studies, scanning electron microscopy was used to characterize the morphotypes of microorganisms present in the root canals of teeth with apical periodontitis (63, 64). While bacteria were found in great numbers in the root canal, in only one specimen they had invaded a short distance beyond the apical foramen. Yeast-like cells were not seen outside the root canal, but in one specimen yeast cells were detected in the middle third of the root canal.

Another approach to investigate the presence of yeasts in the periapical area was taken in a new study where 103 surgically removed periapical granulomas taken from teeth with apical periodontitis resistant to normal conservative endodontic treatment were analyzed (65). After removal of the surface layers (histological sections) from the lesions, the material was subjected to DNA extraction and PCR using specific primers to oral yeasts and controls. DNA could be obtained from 68 lesions and 18 were positive to yeast PCR. However, when the PCR products were analyzed by sequencing, none of the 18 PCR products showed DNA sequence typical for *Candida* spp. Neither could immunohistological examination of these 18 samples show any positive staining indicative of *Candida* infection. The authors concluded that oral yeasts could not be demonstrated in the periapical tissues of the teeth studied (65).

The previous study clearly demonstrated that even with modern molecular biological techniques, the risk of false-positive results must be kept in mind. In the living tissues in the periapical area, 'false positive' can result from cross-reactions, even with specific primers, or by contamination of the sample during extraction or periapical surgical procedures as indicated by Siqueira & Lopes (63). Possible pathways of contamination of the periapical microbiological sample are (i) from the gingival sulcus (intrasulcular incision, (ii) attached gingiva and mucosa (submarginal flap), (iii) sinus tract in the lesion, and (iv) suction of canal contents because of underpressure during operation into the periapical area. Already in 1966, Möller (8) demonstrated the absence of viable microorganisms in resting periradicular inflammatory lesions without sinus tract when the surgical access site was disinfected completely and isolated with the aid of a rubber dam and a customized acrylic shield. Without extreme care, the risk of contamination was evident (8). In two newer studies, bacteriological findings from the periapical area were compared when either full-thickness intrasulcular mucoperiosteal or a submarginal flap was performed in patients with asymptomatic apical periodontitis (61, 66). Both studies showed that fewer bacteria were found from the bone surface samples when a submarginal flap was used. Using the same technique, Sunde et al. (67) analyzed the periapical microflora in 36 teeth with apical periodontitis after 6 months of unsuccessful conservative endodontic treatment. Periapical samples were cultured on non-selective and selective media, including Sabouraud dextrose agar and TSBV plates, both supporting isolation of yeasts. *C. albicans* was isolated from two cases (6%) with no evidence of a sinus tract. Presently, this report is the only one indicating the presence of yeasts in the periapical area. However, it is important to understand the difference between primary (untreated) apical periodontitis and persistent lesions. In the latter, microbes may be pushed into the periapical area during the treatment procedures (iatrogenic extraradicular infection), and while the extruded microorganisms usually cause no or only short-lasting problems (e.g. flare up), it is possible that occasionally densely packed microbial colonies from the root canal may be transferred to the periapical area, where their elimination by host defense can take a long time, or be unsuccessful.

Treatment strategies

Mechanical preparation does not suffice to free a tooth with apical periodontitis from all bacteria in the root canal (68). Furthermore, soft tissue remnants may be left behind after mechanical root canal preparation (69). Collagen and other soft tissue components provide a source of nutrition for microorganisms that survived root canal therapy, allowing them to re-multiply (70). Thus, a so-called 'chemo-mechanical' treatment strategy is advocated with the aid of antimicrobial and tissue-dissolving irrigants to minimize the amount of infected dentine, pulpal remnants, and the number of microorganisms in the root canal system. However, complete disinfection of the root canal system cannot be predictably completed by chemomechanical preparation alone (71). It is currently debated whether an interappointment dressing would further decrease the number of viable microbes in samples from root canal systems. Earlier studies suggested better root canal disinfection after a calcium hydroxide dressing for 1–3 weeks subsequent to instrumentation and irrigation with sodium hypochlorite vs. instrumentation/irrigation alone (72, 73). However, several other studies have found no difference or have indicated even the opposite (74, 75).

Persistent microorganisms, such as *E. faecalis* or *C. albicans*, are often present in root canal infections resistant to conventional therapy. Therefore, attention should be paid to adequate treatment strategies

including selection of medicaments potentially efficient against microorganisms residing in the root canal system. In this context, it should be noted that extended exposure times to microbiota increase the killing efficacy of all antiseptic agents (76). Even fast-acting halogen-releasing agents such as sodium hypochlorite or iodine potassium iodide (IPI) have increased efficacy with extended working time in the root canal (74, 77). Thus, whether the aim is to finish a root canal treatment in one or two visits, adequate time should be given to irrigants or intracanal dressings to achieve proper disinfection.

Irrigating solutions

Sodium hypochlorite is probably the most widely used irrigant in endodontics today. It is a potent disinfectant, killing microorganisms in concentrations between 0.5% and 5%, in clinically relevant periods of time. It has a wide antimicrobial spectrum, and is active against *C. albicans*. Ferguson et al. (78) determined the susceptibility of *C. albicans* to intracanal irrigating solutions and locally used disinfecting medicaments. The minimum inhibitory concentration (MIC) of sodium hypochlorite, hydrogen peroxide, chlorhexidine digluconate, and aqueous calcium hydroxide to inhibit the growth of *C. albicans* was determined. Sodium hypochlorite, hydrogen peroxide, and chlorhexidine digluconate were effective anticandidal agents with MICs of <10, 234, and $<0.63\,\mu g/mL$, respectively. Aqueous calcium hydroxide had no activity. However, when a standardized inoculum of *C. albicans* cells was placed in direct contact with calcium hydroxide paste or camphorated para-monochlorophenol, both were shown to kill the yeast cells effectively.

In addition to antibacterial properties, sodium hypochlorite has a unique potential to dissolve soft tissue, a characteristic not found with other endodontic irrigants (79). However, sodium hypochlorite at high concentrations can be caustic to vital periapical or periodontal tissues, if inadvertently introduced to these (80). It is therefore advocated to irrigate root canals with *high volumes* of a low-concentration solution, i.e. 0.5–1%.

To improve antisepsis in a one-appointment regimen, it has been suggested to rinse/soak the canals with chlorhexidine or IPI solutions following irrigation with sodium hypochlorite (7, 74, 81). Aqueous chlorhexidine solution has a wide-spectrum antimicrobial activity at low concentrations, and is especially effective against *C. albicans* (78). Furthermore, it binds to surrounding tissues to be released again slowly over extended periods of time (82), a phenomenon called substantivity. Interestingly, it appears that chlorhexidine can efficiently inhibit the initial adherence and perhaps further accumulation and biofilm formation of yeasts and other microorganisms (83). A recent clinical study has shown that canals that received a final rinse with a 2% chlorhexidine solution were significantly more often free of cultivable microorganisms than controls irrigated with sodium hypochlorite alone (81). However, chlorhexidine preparations have little to no tissue-dissolving capacity (79), and should therefore only be used after thorough irrigation with sodium hypochlorite. IPI, used as an intravisit dressing for 10 min, has also shown some promise as an endodontic disinfectant. However, iodine preparations may not offer any additional value against yeasts when used in combination with sodium hypochlorite, because they have a similar mode of action. In addition, IPI may stain teeth and is potentially allergenic (84).

Removal of the smear layer appears to improve the antiseptic action of irrigants and intracanal medications (71, 85). EDTA is the most frequently used chelator in root canal therapy (86). Antimicrobial efficacy of EDTA has been described, but to date, the killing efficacy on yeasts in direct exposure tests in comparison with other endodontics irrigants remains unclear (87, 88).

Very little data are available about the effectiveness of various irrigating solutions or disinfecting agents against yeasts *in vivo* in the infected root canal. Peciuliene et al. (7) studied the effect of instrumentation and irrigation in previously root-filled teeth with apical periodontitis. *C. albicans* was found in six of 33 root canals before starting the preparation. A second sample taken after instrumentation and irrigation with 10 mL of 2.5% NaOCl and 5 mL of 17% EDTA revealed no yeasts in any of the canals. In the same study, *E. faecalis* was still present in six canals (originally found in 20 canals) after the chemomechanical preparation described above. The result is an indication that eradication of *C. albicans* from the root canal *in vivo* is an achievable goal with a routine protocol as described.

Disinfecting interappointment medicament: calcium hydroxide

Calcium hydroxide is the interim root canal medication of choice for many endodontists for various reasons:

it has a good antibacterial effect in the infected root canals (72), dissolves necrotic tissue remnants (89), and inactivates endotoxins (90–92). The antimicrobial effects of calcium hydroxide are assumed to be caused by its high pH and high alkaline buffer capacity. Thus, calcium hydroxide is not effective against alkali-resistant microbes such as *C. albicans in vitro*, unless the organisms are in direct contact with calcium hydroxide paste. In a recent report (93), *C. albicans* was found to be even more resistant than *E. faecalis*, which has shown both clinical and *in vitro* resistance against calcium hydroxide (72, 94).

Calcium hydroxide combined with effective aqueous disinfectants may provide an improved interappointment dressing, with an extended antimicrobial spectrum also covering persistent microorganisms such as *C. albicans* (95). It has been shown that combinations of calcium hydroxide with sodium hypochlorite or chlorhexidine solutions maintain the alkaline capacity of aqueous calcium hydroxide suspensions (96), while their efficacy in disinfecting dentinal tubules is improved (97). Sirén et al. (98) have also shown superior disinfecting effect of calcium hydroxide combined with either 0.5% chlorhexidine or 2/4% IPI in dentine blocks infected with *E. faecalis*. While pure calcium hydroxide was shown to be totally ineffective against *E. faecalis* in the dentine, both combination preparations killed *E. faecalis* deep in the dentine during the 1 and 7 days test periods. Although the new combinations were not tested against yeast-infected dentine, the effectiveness of both chlorhexidine and iodine against yeasts alone indicates that the combination products would be more effective against yeasts than pure calcium hydroxide. Interestingly, cytotoxicity experiments showed that the combination products were less cytotoxic than each of the components alone (98). However, more clinical studies are needed to find out whether combining calcium hydroxide with antiseptic irrigants as an interim dressing improves the antimicrobial effectiveness as compared with irrigation with these irrigants followed by a conventional calcium hydroxide dressing.

Antifungal agents

Use of local antibiotic agents in root canal infection has been of interest for decades. However, there is no evidence that antibiotic cocktails would completely disinfect dentine or give higher clinical success rates. Neither is there evidence that local antimycotic substances would give better results than 'traditional' local disinfecting agents. Topical antibiotic agents always cause a local concentration gradient. Microorganisms residing in dentinal tubules or periapical lesions exposed to sublethal concentrations may therefore develop resistance. Therefore, fungal antibiotics should be considered locally and systemically only for the treatment of acute apical periodontitis with severe symptoms after microbiological diagnosis. Intermediate or resistant yeast isolates from infected root canals against azole-group medicaments have already been reported (99).

New approaches in dentine disinfection

Complete disinfection of dentine has shown to be a goal that is difficult to reach with the presently available irrigating solutions and local root canal medicaments. One of the reasons for the poorer *in vivo* effectiveness as compared with *in vitro* results is partial or total inactivation of the antibacterial effect of the various medicaments by dentine and other substances potentially present in the necrotic root canal environment (100–102). Therefore, it sounds logical to search for better disinfecting protocols by combining existing and even new disinfectants, or to find antimicrobial substances that are not affected or are even potentiated by dentine instead of inhibition. Recent reports have indicated the potential of bioactive glass of the SiO_2–Na_2O–CaO–P_2O_5 system for dentine disinfection (103). Bioactive glass alone has a moderate disinfecting potential. Interestingly, the presence of dentine in aqueous suspension appears to boost the antimicrobial activity of bio-active glass. However, the findings were obtained in an *in vitro* environment, and it is premature at this point to draw far-reaching conclusions regarding its clinical effectiveness. Nevertheless, this novel approach to dentine disinfection is promising because of the synergistic and not antagonistic interaction with the host environment.

Conclusions

C. albicans is by far the most common yeast in endodontic infections. Yeasts can be found in low

numbers both from primary infections as well as from post-treatment infections. The number of yeast cells in the root canal is usually much lower than that of bacteria, and it is uncertain at present whether yeasts can survive outside the root canal in the periapical area in 'extraradicular infections'. The ability of *C. albicans* to interact with dentine may be important for its ability to survive in the ecologically demanding environment of the necrotic or treated root canal. Although *C. albicans* and other yeasts are resistant to some locally used disinfecting agents such as calcium hydroxide *in vitro*, there is no solid evidence that their eradication from the root canal is a clinical problem. *C. albicans* is more sensitive to sodium hypochlorite than *E. faecalis* (104), and it is also rapidly killed by low concentrations of chlorhexidine. In the future, combinations of present and new disinfecting agents and substances that may act in a synergistic manner with dentine may take us closer to the goal of complete dentine disinfection.

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